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Liquid Chromatograph Mass Spectrometer LCMS-8045RX

Application News

Quantitative Analysis of QACs in Milk Using a QuEChERs Sample Preparation Approach

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User Benefits

- Sensitive and selective detection of BAC C8–C18 and DDAC C8–C12.
- Limits of quantification suitable for surveillance and compliance testing.
- Fast sample preparation compatible with routine laboratory throughput.

■ Introduction

Quaternary ammonium compounds (QACs) such as benzalkonium chlorides (BACs) and didecyldimethyl-ammonium chloride (DDAC) are widely used cationic disinfectants. Their surface-active, antimicrobial properties make them effective as teat dips, equipment sanitizers, and detergents in dairy farms and dairy processing facilities, but incomplete rinsing or improper application can lead to residues in raw milk and dairy products. Routine, sensitive analysis of QACs in milk is therefore essential to ensure product quality, protect consumers, and maintain regulatory compliance.

This application note demonstrates a robust LC-MS/MS method for the simultaneous determination of common QAC homologues in milk at low ng/mL levels. Under current European guidance, there are no harmonized EU maximum residue limits (MRLs) specifically set for most QAC disinfectants in milk; however, the European Commission and national authorities apply general food safety laws (Regulation (EC) No 178/2002) and contaminants/legislative frameworks requiring that residues are not present as a risk to consumers. National guidance values and tolerance levels have been published in some Member States (typically in the low mg/kg to µg/kg range depending on compound and context), and monitoring is carried out to ensure levels do not interfere with processing (e.g., starter cultures) or pose toxicological concerns.

Fig. 1 Nexera™ X3 and LCMS-8045RX

■ Sample Preparation

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in food testing laboratories. For the described application, a Shimadzu LCMS-8045RX triple-quadrupole mass spectrometer coupled with a Nexera™ X3 UHPLC system was used (Figure 1).

10 g of blank milk were spiked with 100 µL of a standard mixture (BAC-DDAC-Mix 1, HPC Standards GmbH) containing 10 µg/mL resulting in a final concentration of 100 ng BAC-DDAC/mL milk.

Spiked and blank samples were extracted based on a QuEChERS standard procedure[1)] (Figure 2, using RESTEK Q-Sep QuEChERS Extraction Packets, En Method).

For further clean-up, the raw extract was placed into a freezer for 2h. The supernatant was then transferred quickly into a fresh tube leaving the precipitates behind.

Matrix matched calibration standards were prepared by appropriate dilution of the spiked extract with blank extract. Quality control samples were also prepared accordingly using different milk samples.

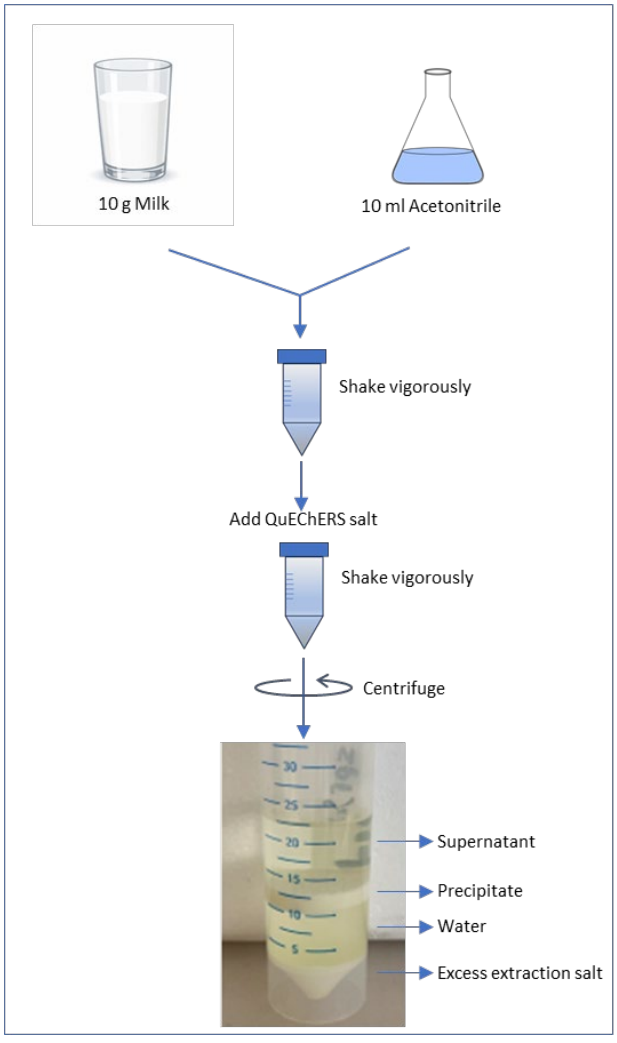

Fig. 2 Flow of Sample Preparation Using QuEChERS

■ Analysis Conditions

Analysis was performed within 12 minutes using MRM acquisition with at least two transitions for each compound.

Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.

Since the presence of QACs is ubiquitous, the use of a solvent delay column is recommended. A small C18 column was placed between the mixer and the autosampler to delay potential QAC contaminations and separate them from sample-derived QACs.

Table 1 Analytical conditions of Nexera and LCMS-8045RX

UHPLC	: Nexera X3
Pump A (Analytical)	: 5 mM ammonium formate + 0.01% formic acid in H_2O
Pump B (Analytical)	: 5 mM ammonium formate + 0.01% formic acid in Methanol
Analytical column	: Shim-pack Velox SP-C18 (100 mm × 2.1 mm I.D., 2.7 µm)*1
Delay column	: Shim-pack GIST HP C18-AQ (30 mm × 3 mm I.D., 3 µm)*2
Injection Volume	: 1 µL
Cooler temperature	: 15 ˚C
Column Oven	: 40 ˚C

Mass Spectrometer	: LCMS-8045RX
Ionization	: Electrospray Ionization (ESI), positive
Interface Voltage	: 0.5 kV
Focus Voltage	: 1 kV
Heating Gas	: 12 L/min
DL Temp.	: 200 ˚C
Interface Temp.	: 400 ˚C
Nebulizing Gas	: 2 L/min
Drying Gas	: 4 L/min
Heat Block	: 400 ˚C
CID	: 270 kPa

*1 P/N: 227-32003-03, *2 P/N: 227-30766-01

■ Results

Matrix-matched calibration curves were determined in duplicate and calculated using weighted (1/conc) linear regression with R^2 >0.99 for all QACs. Exemplary calibration curves and a MRM-chromatogram at 2.5 ng/mL are presented in Figure 3 and Figure 4. Results of quality control samples are shown in Table 3.

As small peaks of DDAC, BAC-C12 and BAC-C14 appear to be consistently present, the LOQ was set to 2.5 ng/mL (2.5 µg/kg) for the individual QACs.

Because no specific maximum residue limits of DDAC and BAC have been established, the general residue limit of

0.01 mg/kg applies. The achieved limits of quantification are well below 0.01 mg/kg (10 ng/mL).

Table 2 MRM transitions and calibration information

Compound	RT	Quantifier	Qualifier	Concentrations	Unit	R^2
DDAC-C12	6.527	382.50>214.30	382.50>58.10	2.5 - 100	ng/mL	0.9998837
BAC-C16	5.963	360.40>91.00	360.40>268.30	2.5 - 100	ng/mL	0.9998196
DDAC-C10	5.577	326.40>186.30	326.40>57.10	2.5 - 100	ng/mL	0.9998912
BAC-C18	6.488	388.50>91.20	388.50>296.20	2.5 - 100	ng/mL	0.9998867
BAC-C8	2.933	248.30>91.05	248.30>156.10	2.5 - 100	ng/mL	0.9998083
BAC-C10	3.676	276.40>91.00	276.40>184.10	2.5 - 100	ng/mL	0.9997886
BAC-C14	5.316	332.40>91.00	332.40>240.20	2.5 - 100	ng/mL	0.9997896
BAC-C12	4.539	304.40>91.00	304.40>212.20	2.5 - 100	ng/mL	0.9994769
DDAC-C8	4.209	270.40>158.10	270.40>58.10	2.5 - 100	ng/mL	0.9998293

Table 3 Results Quality Control samples

		BAC-C8		BAC-C10		BAC-C12		BAC-C14		BAC-C16		BAC-C18	
Sample Name		**Conc. (ng/mL)**	**Accuracy (%)**	**Conc. (ng/mL)**	**Accuracy (%)**	**Conc. (ng/mL)**	**Accuracy (%)**	**Conc. (ng/mL)**	**Accuracy (%)**	**Conc. (ng/mL)**	**Accuracy (%)**	**Conc. (ng/mL)**	**Accuracy (%)**
QC 7.5 ng_mL	**Whole Milk**	6.83	91.01	7.04	93.93	6.76	90.16	6.83	91.07	7.12	94.89	7.21	96.09
QC 7.5 ng_mL	**Organic Milk**	6.29	83.84	6.04	80.53	6.01	80.17	6.00	79.94	6.61	88.14	6.43	85.67
QC 7.5 ng_mL	**Alpine Milk**	6.80	90.67	6.84	91.16	6.92	92.33	6.74	89.85	7.07	94.29	6.95	92.62
Mean		6.64		6.64		6.57		6.52		6.93		6.86	
SD		0.30		0.53		0.49		0.46		0.28		0.40	
%RSD		4.57		7.99		7.41		7.02		4.04		5.80	
QC 30 ng_mL	**Whole Milk**	27.02	90.06	27.02	90.07	26.63	88.78	26.78	89.26	27.22	90.72	27.15	90.49
QC 30 ng_mL	**Organic Milk**	27.02	90.07	26.54	88.48	26.54	88.48	26.22	87.42	26.24	87.45	26.46	88.21
QC 30 ng_mL	**Alpine Milk**	25.92	86.41	25.72	85.72	26.39	87.98	25.67	85.56	25.61	85.37	25.08	83.61
Mean		26.65		26.43		26.52		26.22		26.35		26.23	
SD		0.63		0.66		0.12		0.55		0.81		1.05	
%RSD		2.38		2.50		0.46		2.11		3.07		4.00	
QC 80 ng_mL	**Whole Milk**	72.01	90.02	71.84	89.80	70.63	88.29	70.66	88.33	71.68	89.61	73.66	92.07
QC 80 ng_mL	**Organic Milk**	65.49	81.86	64.09	80.11	64.01	80.01	63.13	78.92	63.15	78.94	63.50	79.38
QC 80 ng_mL	**Alpine Milk**	70.01	87.51	68.56	85.70	71.14	88.93	69.41	86.77	68.59	85.74	68.83	86.04
Mean		69.17		68.17		68.60		67.74		67.81		68.66	
SD		3.34		3.89		3.98		4.04		4.32		5.08	
%RSD		4.83		5.71		5.80		5.96		6.37		7.40	

Table 3 Results Quality Control samples (continued)

Sample Name		DDAC-C8		DDAC-C10		DDAC-C12	
		Conc. (ng/mL)	Accuracy (%)	Conc. (ng/mL)	Accuracy (%)	Conc. (ng/mL)	Accuracy (%)
QC 7.5 ng_mL	Whole Milk	6.87	91.62	6.77	90.22	7.29	97.16
QC 7.5 ng_mL	Organic Milk	6.10	81.32	6.28	83.68	6.55	87.32
QC 7.5 ng_mL	Alpine Milk	6.83	91.02	6.88	91.69	7.10	94.72
Mean		6.60		6.64		6.98	
SD		0.43		0.32		0.38	
%RSD		6.57		4.82		5.51	
QC 30 ng_mL	Whole Milk	27.05	90.17	27.78	92.61	27.86	92.85
QC 30 ng_mL	Organic Milk	26.63	88.76	27.38	91.26	27.13	90.44
QC 30 ng_mL	Alpine Milk	25.94	86.46	25.96	86.54	26.29	87.64
Mean		26.54		27.04		27.09	
SD		0.56		0.96		0.78	
%RSD		2.11		3.54		2.89	
QC 80 ng_mL	Whole Milk	71.55	89.44	70.47	88.08	73.12	91.41
QC 80 ng_mL	Organic Milk	64.08	80.10	64.09	80.12	63.28	79.10
QC 80 ng_mL	Alpine Milk	69.51	86.88	68.65	85.81	70.63	88.29
Mean		68.38		67.74		69.01	
SD		3.86		3.28		5.12	
%RSD		5.64		4.85		7.41	

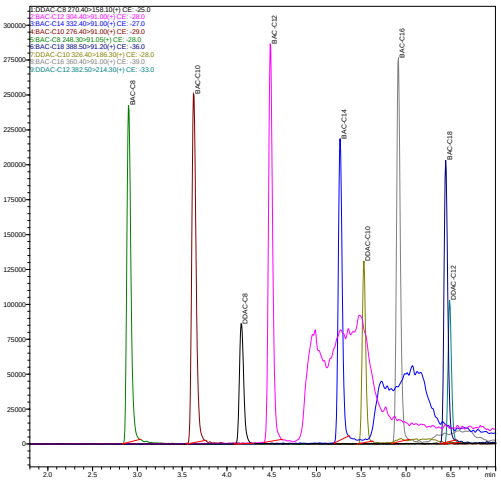

Figure 3 Typical chromatogram at 2.5 ng/mL

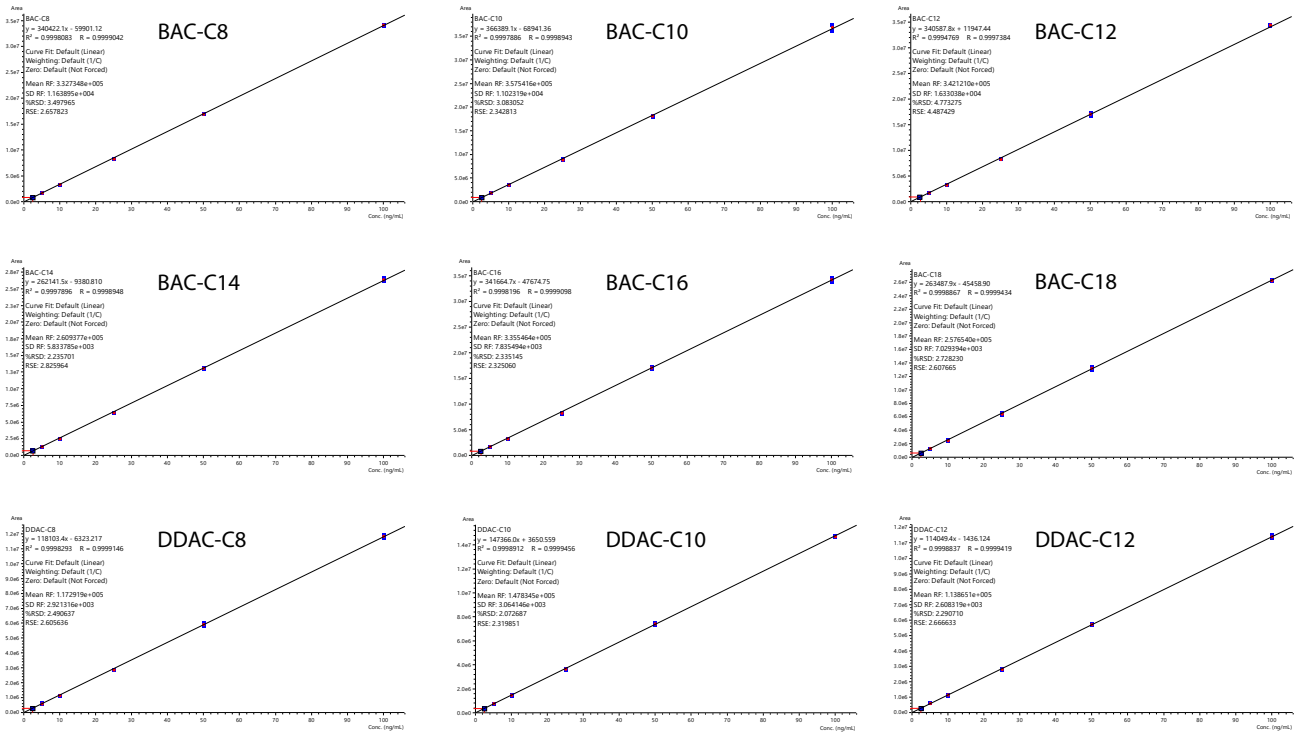

Figure 4 Calibration curves of all QACs

■ Conclusions

This application note describes an LC-MS/MS method utilizing a QuEChERS sample preparation approach to monitor 9 QACs in milk matrix. This proof of concept study using the LCMS-8045RX coupled with a Nexera UHPLC system demonstrates a sensitive method for QAC analysis in milk matrix with minimal sample preparation steps.

■ References

1) Analysis of BACs and DDAC in Milk using QuEChERS method and LC-MS/MS, Version 2, EU Reference Laboratory for Pesticides Requiring Single Residue Methods, CVUA Stuttgart, Schaflandstr. 3/2, 70736 Fellbach, Germany

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